Remarks/Arguments:

Claims 13-60 are pending in the application. Claims 13-19, 21-27, 35-37, and 44-46 stand withdrawn. Claims 20, 28-34, 38-43, and 47-60 were examined and stand rejected as described below. Applicants have not amended any claims concurrent with this response.

I. Rejection over Andersen *et al.* in view of Chaturvedi *et al.*

Claims 20, 28, 29; 38, and 47-55 stand rejected under 35 U.S.C. 103(a) as allegedly obvious over Andersen *et al.* (U.S. 2002/0094336); in view of Chaturvedi *et al.* (Vaccine 17:2882-2887; 1999). Applicants disagree with the rejection.

The examined claims relate to an immunotherapeutic agent and pharmaceutical compositions comprising the same. Due to the difficulties in characterizing the immunotherapeutic agent, it has been claimed in product-by-process claims format. Despite this, the Office Action cited to MPEP 2113:

> "[E]ven though product-by-process claims are limited by and defined by the process, determination of patentability is based on the product itself. The patentability of a product does not depend on its method of production. If the product in the product-by-process claim is the same as or obvious from a product of the prior art, the claim is unpatentable even though the prior product was made by a different process."

However, MPEP 2113 further states:

> "The structure implied by the process steps should be considered when assessing the patentability of product-by-process claims over the prior art, especially where the product can only be defined by the process steps by which the product is made, or where the manufacturing process steps would be expected to impart distinctive structural characteristics to the final product."

It appears, however, that the Office has considered the claimed immunotherapeutic agent as only comprising cell wall fragments from a virulent *Mycobacterium tuberculosis* H37Rv

strain. However, the immunotherapeutic agent of the instant invention is obtainable by a process comprising the steps of (emphasis added):

a) culturing the cells of a virulent *Mycobacterium tuberculosis* - complex (MTB-C) strain for a period of at least three weeks and

b) homogenizing the cells in the presence of a non-ionic surfactant to produce a homogenate comprising non-fragmented cells, cell wall fragments, and solubilized cell compounds.

As would be appreciated by a person having ordinary skill in the art, the steps and conditions of the process (*i.e.*, the culture period, and the fact that the homogenization is carried out in the presence of a non-ionic surfactant) will impart distinctive structural characteristics to the final product (*e.g.,* the non-ionic surfactant is expected to form micelles and solubilize part of the cell components including some components of the cell wall and some antigens).

Furthermore, the immunotherapeutic agent of the invention has been found to be suitable for treating tuberculosis. In particular, it has been found unexpectedly to have a synergistic activity when given as a combined treatment with drugs (*e.g.*, isoniazid or rifampicin) for the treatment of tuberculosis (paragraph [0047] and section IV of Example 3, and in particular, table 6). Advantageously, due to this synergistic activity, the immunotherapeutic agent of the instant invention would reduce the time of treatment with these drugs (paragraph [0118]) and thus also reduce the risk of developing resistance to the drugs. This is highly desirable, taking into account that, prior to the instant invention, the period of treatment of tuberculosis with these drugs was several months in length (paragraphs [0006] and [0007]).

The claims are not obvious over the Anderson or Chatuvedi references cited by the Office Action, either taken alone or in combination.

Andersen *et al.* describes immunologically active polypeptide fragments which comprise a sequence derived from the sequences of more than 30 identified antigens and putative antigens (paragraphs [0014] to [0018]), a fusion polypeptide, DNA fragments encoding such polypeptides, a vaccine effective against tuberculosis comprising said nucleic acid fragments (paragraph [0089]), an immunologic composition comprising the mentioned polypeptides

(paragraph [0092]) and diagnostic compositions comprising said polypeptides for the detection of infection with mycobacteria.

The reason why the polypeptide fragments are excellent candidates for vaccine constituents is explained in paragraph [0091]:

> "[0091] As explained above, the polypeptide fragments of the invention are excellent candidates for vaccine constituents or for constituents in an immune diagnostic agent due to their extracellular presence in culture media containing metabolizing virulent mycobacteria belonging to the tuberculosis complex, or because of their high homologies with such extracellular antigens, or because of their absence in M. bovis BCG."

Among the more than 30 antigens of *M. tuberculosis* identified in Andersen *et al.* (paragraphs [0014] to [0018]), most of them from a short term culture filtrate, only one single antigen is stated to have been isolated from the cell wall (*i.e.*, CWP32, see paragraphs [0289] and [0290]). Furthermore, the process for isolating the antigen does not comprise the key steps of the process for preparing the immunotherapeutic agent of the instant invention. The antigen CWP32 is described to have been isolated (paragraph [0290]) from a cell wall fraction of H37Rv prepared as described in Sørensen *et al.*, Infect. Immun., 1995, 1710-1717 (Exhibit A).

The process for preparing the cell wall fraction disclosed in Sørensen *et al.* (page 1711, right column, paragraph Subcellular fractionation of *M. tuberculosis*) comprises the following steps (emphasis added):

a) Harvesting seven-day-old cultures of *M. tuberculosis* H37Rv by centrifugation, washing them in cold PBS, and recovering a wet pellet.
b) Resuspending the wet pellet in a lysis buffer consisting of PBS supplemented with the protease inhibitor phenylmethylsulfonyl fluoride and EDTA.
c) Lysing the cells with a French pressure cell at 12,000 lb/in^2.
d) Removal of bacterial cells resisting lysis treatment by low-speed centrifugation.
e) Centrifugation of supernatant at 20,000 x g to obtain a pellet, which was assumed to consist mainly of cell walls.

Thus, the process used for preparing the cell wall fraction disclosed in Sørensen *et al.* differs from the process for preparing the immunotherapeutic agent of the instant invention in that:

- the culture period is of seven days instead of at least three weeks,
- the lysis of the cells is not performed in the presence of a non-ionic surfactant.

Consequently, Applicants submit that it is apparent the immunotherapeutic agent obtained according to the process of the instant application must be different in structure and properties from the cell wall extract prepared according to the process disclosed in the document Sørensen *et al.* referenced by Andersen.

Neither Andersen nor the document it refers to (Sørensen *et al.*) discloses a process for preparing an immunotherapeutic agent by a process comprising the steps of the instant invention,

a) culturing the cells of a virulent *Mycobacterium tuberculosis* - complex (MTB-C) strain for a period of at least three weeks and

b) homogenizing the cells in the presence of a non-ionic surfactant to produce a homogenate comprising non-fragmented cells, cell wall fragments, and solubilized cell compounds.

Furthermore, neither Andersen nor the document it refers to (Sørensen *et al.*) suggest, or motivate the person having ordinary skill in the art, to modify the process for preparing the cell wall fraction as claimed, since modifying the culture period and performing the lysis of the cells in the presence of a non-ionic surfactant must give a final product with a different composition and thus different properties.

Chaturvedi *et al.* does not cure these deficiencies. Regarding Chaturvedi *et al.*, the Office Action appears to have misinterpreted the teachings of this reference, stating that:

> - "Chaturvedi et al. describe the preparation of protective antigens from the cell wall of Mycobacterium habana (Title and Abstract), by sonication, differential centrifugation, phase separation using Triton X-114, centrifugation, backwashing in buffer having a neutral pH, and recovery of protein fractions by precipitation and lyophilization (sections 2.1 and 2.3 p. 2883; limitation of claims 28 and 51)."

> - "The authors present results from cell wall fraction vaccination against M. tuberculosis H37Rv, and state that the strain is a good choice as a candidate vaccine for tuberculosis (first column, p. 2887)."

But, the strain that is stated to be a good choice as a candidate vaccine for tuberculosis in Chaturvedi *et al.* is not the virulent H37Rv. On the contrary, the strain is *Mycobacterium habana*, which is a cultivable nonpathogenic mycobacterium:

> "Interestingly, separation pattern of peripheral and integral membrane proteins is same as that of *M. tuberculosis* H37Rv [12] which puts *M. habana* more close to *M. tuberculosis* and makes it a good choice as candidate vaccine for tuberculosis."
> (page 2887, last sentence of last paragraph)

Moreover, the reason why it is recommended as a vaccine is mainly due to its membrane proteins.

Chaturvedi describes the preparation of protective antigens from different subcellular fractions of *Mycobacterium habana* including the cell wall (see, Abstract). However, the preparation of the cell wall fraction does not include a phase separation using Triton X-114, as suggested by the Office Action. The phase separation using Triton X-114 is performed instead over the membrane fraction of *Mycobacterium habana* (sections 2.1 and 2.3 p. 2883). The cell wall fraction is prepared merely by sonication and differential centrifugation.

In Chaturvedi, the extraction with Triton X-114 is performed to separate the proteins of peripheral compartment of the membrane from those associated with the core of lipid bilayer belonging to integral compartment of the membrane and further the residual insoluble material is discarded. Thus page 2883, section 2.3, beginning of first paragraph reads:

> "2.3. Separation of proteins of integral and peripheral compartments of the membrane fraction
> Proteins of peripheral compartment of the membrane were separated from those associated with the core of lipid bilayer belonging to integral compartment [21,22] by phase separation using Triton X-114 [11,12]. Briefly, to a suspension of purified membrane pellet, 10 mg protein/ml

> Tris buffer (10 mM Tris-HCl, pH 7.4 containing 150 mM NaCl and 0.02% NaN_3) 50 ml of precondensed [23] Triton X-114 (in Tris buffer) was added. The solution was mixed and chilled in ice to make a single phase. Residual insoluble material was removed by centrifugation at 2500 x g for 10 min at 37°C."

The cell wall fraction in Chaturvedi (section 2.1, p. 2883) is prepared as described in a previous article from the same main author, in Chaturvedi *et al.*, *Lepr Rev* (1995) 66, 31-38 (Exhibit B).

The process for preparing the cell wall fraction disclosed in Chaturvedi *et al.*, *Lepr Rev* (1995) 66, 31-38 (page 32, subsections Growth of *Mycobacterium habana* and Fractionation of *M. habana*) includes the following steps (emphasis added):

a) Harvesting 10-day-old cultures of *M. habana* by centrifugation and washing 3 times with normal saline.
b) Suspending the harvest in Tris-buffered saline (TBS; 0.01 M Tris, pH 7.4) containing $MgCl_2$ (10 mM) and protease inhibitors (1 mM).
c) Lysing the cells by sonication for 10 min at >20k cycles s^{-1} in phasic manner (50% s^{-1}).
d) Centrifuging the sonicate at 15,000 x g for 30 min at 4 °C to isolate the cell wall (pellet) and washing it three times with TBS.

Thus, the process used for preparing the cell wall fraction disclosed in Chaturvedi *et al.*, *Lepr Rev* (1995) 66, 31-38 differs from the process for preparing the immunotherapeutic agent of the instant invention in the key features:

- the culture period is of 10 days instead of at least three weeks,
- the lysis of the cells is not performed in the presence of a non-ionic surfactant.

Consequently, Applicants submit that it is apparent the immunotherapeutic agent obtained according to the claimed process steps must be different in structure and properties from the cell wall extract prepared according to the process disclosed in the document Chaturvedi *et al.*, *Lepr Rev* (1995) 66, 31-38 referenced to in Chaturvedi *et al.*

In summary, Chaturvedi does not disclose or suggest the preparation of protective antigens from a cell wall fraction of the virulent strain H37Rv, but instead from the non-

pathogenic strain *Mycobacterium Habana*. Chaturvedi *et al.* does not disclose or suggest the preparation of the cell wall fraction by a process comprising a phase extraction with a non-ionic surfactant. Furthermore, Chaturvedi *et al.*, discourages the use of the obtained cell wall fraction for preparing a vaccine against tuberculosis since it is stated to induce a lower survival rate in immunized mice than the membrane fraction (cf. page 2885, section 3.3, second paragraph) and it is acknowledged that the protective effect observed for the cell wall fraction may have been partly due to contamination with antigens from the membrane (page 2886, paragraph bridging first and second column).

It is respectfully submitted that the teaching of Andersen *et al.* and Chaturvedi *et al.*, taken alone or in combination, would not have motivated the person of ordinary skill in the art to prepare an immunotherapeutic agent by isolating cell wall fragments from a virulent *Mycobacterium tuberculosis* - complex (MTB-C) strain comprising a step of homogenizing the cells in the presence of a non-ionic surfactant, as instantly claimed, with a reasonable expectation of success, at the time of the instant invention, since modifying the culture period and performing the lysis of the cells in the presence of a non-ionic surfactant yields a final product with a different composition and thus different properties.

Accordingly, withdrawal of the rejection of claims 20, 28, 29; 38, and 47-55 under 35 U.S.C. 103(a) as obvious over Andersen *et al.* in view of Chaturvedi *et al.* is respectfully requested.

II. Rejection over Andersen *et al.* in view of Chaturvedi *et al.* and further in view of Unger *et al.*

Claims 28-33, 38-42, 47, 54 and 56-59 stand rejected under 35 U.S.C. 103(a) as allegedly obvious over Andersen *et al.* (U.S. Patent Application Publication No.: 2002/0094336), in view of Chaturvedi *et al.*, as applied to claims 20, 28, 29, 38, and 47-55 above, and further in view of Unger *et al.* (U.S. Patent No.: 6,443,898). According to the Office Action, these claims embrace an immunotherapeutic agent containing cell wall fragments from a virulent *Mycobacterium tuberculosis* H37Rv strain of cells and a pharmaceutical composition comprising the same in the form or liposomes.

It appears that the Office has considered the claimed immunotherapeutic agent as being only characterized by comprising cell wall fragments from a virulent *Mycobacterium tuberculosis*

H37Rv strain. As explained above, however, the immunotherapeutic agent of the instant invention is not only characterized by containing cell wall fragments from a virulent *Mycobacterium tuberculosis* - complex (MTB-C) strain, but also by the features imparted by the process of manufacture:

a) culturing the cells of for a period of at least three weeks and

b) homogenizing the cells in the presence of a non-ionic surfactant to produce a homogenate comprising non-fragmented cells, cell wall fragments, and solubilized cell compounds.

The person having ordinary skill in the art will recognize that these steps of the process will impart distinctive structural characteristics to the final product

Neither Andersen nor Chaturvedi, taken alone or in combination, as discussed in detail above, teaches the preparation of an immunotherapeutic agent containing cell wall fragments by a process comprising a step of homogenizing the cells in the presence of a non-ionic surfactant, as instantly claimed. Furthermore, neither Andersen nor Chaturvedi, taken alone or in combination, as discussed in detail above, suggest or motivates the skilled artisan to prepare an immunotherapeutic agent by including such step, since it is apparent that the consequence of performing such step renders an immunotherapeutic agent containing a cell wall extract of different composition and thus different properties.

Unger *et al.* does not cure these deficiencies, since Unger neither teaches nor suggests any method for preparing cell wall fragments. Unger merely describes therapeutic delivery systems comprising gaseous precursor-filled liposomes comprising a therapeutic agent (Abstract) and include among more than 200 hundred suitable therapeutic agents, microbial cell wall components and sub-units of bacteria (such as Mycobacteria, Corynebacteria), mentioned in a completely general way.

The Office has relied on Unger *et al.* for the feature that the pharmaceutical composition is in the form of liposomes. Since, as discussed above, claims 20, 28, 29; 38, and 47-55 are non-obvious over Andersen *et al.* in view of Chaturvedi *et al.*, then dependent claims 30-33, 39-42 and 56-59, which further limit the composition to be in the form of liposomes, must also be non-obvious. For at least these reasons, the Applicants respectfully request withdrawal of the rejection.

III. Rejection over Andersen *et al.* in view of Chaturvedi *et al.* and further in view of Unger *et al.* and Parikh.

Claims 28, 38, 39, 43, 47, 54 and 56-60 stand rejected under 35 U.S.C. 103(a) as allegedly obvious over Andersen *et al.* (U.S. Patent Application Publication No.; 2002/0094336); in view of Chatuverdi *et al.* (Vaccine 17:2882-2887; 1999), and further in view of Unger *et al.* (US. Patent No.: 6,443,898) as applied to claims 28-33, 38-42, 47, 54 and 56-59 above, and Parikh. (U.S. Patent No.: 5,785,975).

According to the Office Action, these claims embrace an immunotherapeutic agent containing cell wall fragments from a virulent *Mycobacterium tuberculosis* H37Rv strain of cells and a pharmaceutical composition comprising the same in the form or Iiposomes, further comprising vitamin E. It appears, however, that the Office has considered the claimed immunotherapeutic agent as being only characterized by comprising cell wall fragments from a virulent *Mycobacterium tuberculosis* H37Rv strain.

As explained above, the immunotherapeutic agent of the instant invention is not only characterized by containing cell wall fragments from a virulent *Mycobacterium tuberculosis* - complex (MTB-C) strain, but also by the features implied by the process of manufacture:

a) culturing the cells of for a period of at least three weeks and

b) homogenizing the cells in the presence of a non-ionic surfactant to produce a homogenate comprising non-fragmented cells, cell wall fragments, and solubilized cell compounds.

The person having ordinary skill in the art will recognize that these steps of the process will impart distinctive structural characteristics to the final product

Neither Andersen nor Chaturvedi nor Unger, taken alone or in combination, as discussed in detail above, teaches the preparation of an immunotherapeutic agent containing cell wall fragments by a process comprising a step of homogenizing the cells in the presence of a non-ionic surfactant, as instantly claimed. Furthermore, neither Andersen nor Chaturvedi nor Unger, taken alone or in combination, as discussed in detail above, suggest or motivates the person having ordinary skill in the art to prepare an immunotherapeutic agent by including such a step, since it is apparent that the consequence of performing such a step is rendering an

immunotherapeutic agent containing a cell wall extract of different composition and thus different properties.

Parikh does not cure this deficiency, since Parikh neither teaches nor suggests any method for preparing cell wall fragments. Parikh merely describes phospholipid adjuvant compositions and vaccine formulations (Abstract), stating that examples of vehicles with adjuvant-like activities include water/oil emulsions, oil/water emulsions, microencapsulation, and liposomes (paragraph 15), and in Example II, disclose a vaccine emulsion formulation comprising a mixture of beta-glucanphospholipid conjugate, phosphatidylcholine and vitamin E (paragraph 44).

The Office has relied on Parikh for the feature that the pharmaceutical composition in the form of liposomes further comprises vitamin E. Since, as discussed above, claims 28-33, 38-42, 47, 54 and 56-59 are non-obvious over Andersen *et al.* in view of Chaturvedi *et al.* and further in view of Unger *et al.*, then dependent claims 34, 43 and 60, which further limit the composition to comprise vitamin E, must also be non-obvious. For these reasons, the Applicants respectfully request withdraw of the rejection.

IV. Concluding remarks

The immunotherapeutic agent of the claimed invention is not only characterized by containing cell wall fragments from a virulent *Mycobacterium tuberculosis* - complex (MTB-C) strain, but also by the features implied by the process of manufacture:

a) culturing the cells of for a period of at least three weeks and

b) homogenizing the cells in the presence of a non-ionic surfactant to produce a homogenate comprising non-fragmented cells, cell wall fragments, and solubilized cell compounds.

It is apparent for a person having ordinary skill in the art that these steps of the process will impart distinctive structural characteristics to the final product (*e.g.*, the non-ionic surfactant is expected to form micelles and solubilize part of the cell components including some components of the cell wall and some antigens). None of the references cited by the Office Action, taken alone or in combination, teaches the preparation of an immunotherapeutic agent containing cell wall fragments by a process comprising a step of homogenizing the cells in the presence of a non-ionic surfactant, as instantly claimed. Consequently, Applicants

respectfully submit that it is apparent that the immunotherapeutic agent obtained according to the process of the instant application must be different in structure and properties from the cell wall extract prepared according to the process disclosed in the references.

Furthermore, none of the documents quoted by the Office, taken alone or in combination, suggest or motivates the skill person having ordinary skill in the art to prepare an immunotherapeutic agent by including such step, since it is apparent that the consequence of performing such step is rendering an immunotherapeutic agent containing a cell wall extract of different composition and thus different properties. And, none of the references provide any reason that would have made obvious, at the time of the invention, the unexpected synergistic effect of the immunotherapeutic agent of the instant invention observed when given in combination with other drugs suitable for the treatment of tuberculosis (paragraph [0047] and section IV of Example 3, in particular, table 6).

Applicants respectfully request reconsideration and withdrawal of the rejections in light of the arguments set forth herein, and solicit a Notice of Allowance of all pending claims.

Respectfully submitted,

Jacques L. Etkowicz, Reg. No. 41,738
Brian A. Cocca, Ph.D., Reg. No. 58,583
Attorneys for Applicants

Attachments: Exhibit A: Sørensen et al., Infect. Immun., 1995, 1710-1717.
Exhibit B: Chaturvedi et al., Lepr Rev (1995) 66, 31-38.

Dated: June 4, 2009

☒ P.O. Box 980
Valley Forge, PA 19482
(610) 407-0700

The Director is hereby authorized to charge or credit Deposit Account No. **18-0350** for any additional fees, or any underpayment or credit for overpayment in connection herewith.